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Structure-Genotoxic Activity Relationships of Pesticides Comparison of the Results from Several Short-Term Assays

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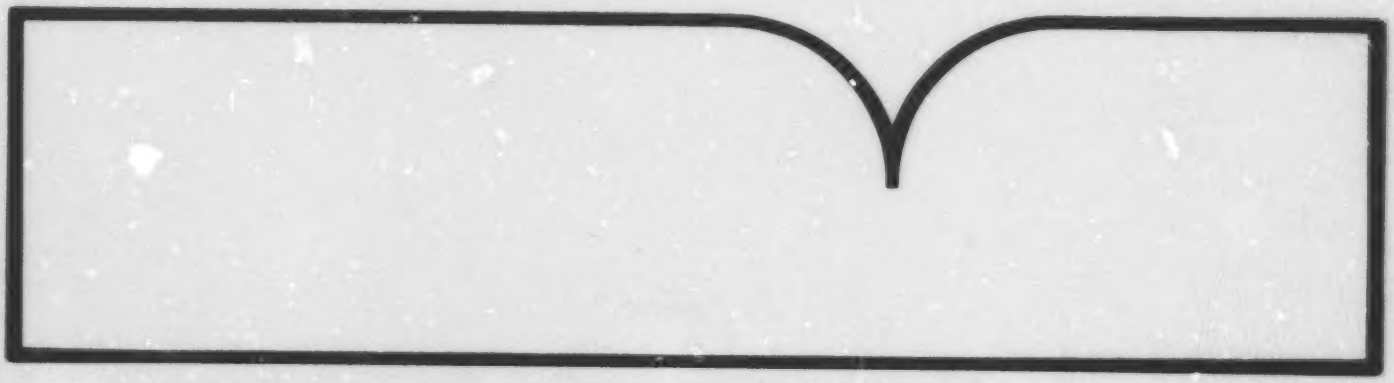

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Among the most relevant fragments, automatically selected by the program, a methoxyphosphinyl and a chloro-vinyl group appear as the common structural subunits responsible for the activities detected in the battery composed of the Salmonella typhimurium mutagenicity, the Mouse Lymphoma gene mutation assay and recombinogenicity in the yeast Saccharomyces cerevisiae.

17. KEY WORDS AND DOCUMENT ANALYSIS

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Structure–genotoxic activity relationships of pesticides: comparison of the results from several short-term assays

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Summary

The Computer-Automated Structure Evaluation (CASE) program has been applied to the analysis of the genotoxic activity of 54 pesticides (31 insecticides, 15 herbicides and 8 fungicides) in 5 different short-term test systems measuring gene mutation and DNA damage. The database contains compounds presenting diverse structures including carbamates, thiocarbamates, organophosphates, halo-aromatics and other functionalities. Some significant relationships between common structural features and the genotoxic activity displayed by these chemicals have been found.

Among the most relevant fragments, automatically selected by the program, a methoxyphosphinyl and a chlorovinyl group appear as the common structural subunits responsible for the activities detected in the battery composed of the *Salmonella typhimurium* histidine reversion assay, the mouse lymphoma gene mutation assay and recombination in the yeast *Saccharomyces cerevisiae*.

Among the potential hazardous effects of agricultural chemicals, carcinogenesis is of special concern. For this reason, the genotoxic and mutagenic activities of a number of pesticides have been the object of extensive research. These activities have predictive value for the potential of the pesticide to cause cancer (Innes et al., 1969; Mahr and Miltenburger, 1976; Simmon, 1978; Waters et al., 1980; Moriya et al., 1983; Ames et al., 1975; Rosenkranz et al., 1984). As a result, appreciable experimental data have been generated, making possible a preliminary study of the relationships between genotoxic and mutagenic properties and chemical structure.

Heretofore, pattern-recognition (Jurs et al., 1983) and branching analysis (Mager, 1981) have been performed in attempts to correlate chemical structure and the genotoxic activity of pesticides. Recently, Jurs et al. (1983) have reported both quantitative and qualitative studies on the mutagenic behavior of some pesticides and other compounds in short-term test systems. These authors have analyzed a number of pesticides in the *Saccharomyces cerevisiae* mitotic recombination assay. 9 descriptors were required to identify correctly the activity of the 42 chemicals considered.

To compare the ability of selected short-term assays to detect and confirm genetic activities of pesticides, Waters et al. (1982) have studied extensively in different test systems the genotoxic activity displayed by a number of pesticides presenting diverse chemical structures. The short-term bioassays assessed 3 types of genetic damage: (1) gene or point mutations, (2) primary DNA damage and

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repair, and (3) chromosomal alterations.

The purpose of this communication is to report the results, using a new artificial intelligence methodology (CASE) to predict structure–activity relationships, of an analysis of the structural determinants of the genotoxic activity of 54 of the 63 pesticides studied by Waters et al. (1982). For this study, 5 short-term test systems measuring gene mutation and DNA damage in prokaryotes and eukaryotes were selected for analysis. Special attention was devoted to the identification of the most relevant structural features responsible for activity or inactivity for each endpoint and to the recognition of possible common structural factors causing a chemical to be active or inactive in a given battery of short-term assays. Because of the chemical diversity of the pesticides under consideration, it was hoped that CASE would provide some important clues concerning the specificity of the observed mutagenic and genotoxic effects.

Method

The CASE program

The CASE program has been described previously (Klopman, 1984; Klopman and Rosenkranz, 1984; Klopman and Contreras, 1984) so, it is discussed only briefly here. The input to the program consists of the molecular structures and a digit describing the genetic activity for each molecule. Potencies are not available at present, so chemicals are labeled simply as inactive or active. Structural information is entered in the computer using a simple line notation: the KLN code previously described by Klopman and McGonigal (1981). The code is similar to the Wiswesser Line Notation, but is better adapted to the CASE program for coding a variety of molecular structures rapidly.

Once this information has been entered, the program proceeds to generate all the possible fragments resulting from breaking the molecules into subunits containing from 3 to 10 heavy atoms with their attached hydrogens. Each fragment is labeled as activating or inactivating if it belongs to an active or inactive molecule. A statistical analysis is performed and, based on probability criteria, significant molecular substructures are selected automatically.

The current version of the program has an additional capability that allows comparisons to be made between different databases to identify possible commonalities in the fragments that cause a compound to be active or inactive in a variety of biological assays. This new capability is of special interest to the present study, because it addresses the problem of designing a sensitive battery of short-term assays that enhances the probability of detecting relevant activity while minimizing the time and expense involved in the screening process (Waters et al., 1982).

Results and discussion

In Table 1, the common names of the 54 pesticides under analysis are presented with their structural formulae. Compounds 1–31 are insecticides, compounds 32–46 herbicides, and compounds 47–54 fungicides. Table 1 also summarizes the activity of the test chemicals in 5 short-term assays: the *Salmonella typhimurium* histidine reversion assay (SAL); the thymidine kinase gene mutation assay using mouse lymphoma L5178Y cells (L5T); prokaryotic DNA repair assays using, respectively, pairs of *Escherichia coli* polA$^+$ and polA$^-$ (REP) and *Bacillus subtilis rec*$^+$ and *rec*$^-$ (REW) strains; and mitotic recombination in strain D3 of the yeast *Saccharomyces cerevisiae* (YE3). The details of the assays have been described previously by Waters et al. (1982).

Test results were summarized as either positive, negative or 'no test'; the latter designation was used for ambiguous results obtained in REP and REW.

The first step in our analysis was to translate compounds numbered 1–54 into KLN code and label them active or inactive according to their responses in YE3. Only those fragments found in distributions that would be observed with less than 15% probability if their occurrence was random (i.e., more than 85% confidence level that they were causally related to activity or inactivity) were retained for further analysis. 4 activating (fragments I–IV) and 2 inactivating fragments (V and VI) qualified under this constraint. Fig. 1 shows these fragments and the probability that their distribution among active and inactive molecules is not due to chance alone.

TABLE 1

COMMON AND CHEMICAL NAMES, CAS NUMBERS AND EXPERIMENTRAL ACTIVITIES DISPLAYED IN THE YE3, SAL, L5T, REW AND REP SHORT-TERM BIOASSAYS OF PESTICIDES UNDER ANALYSIS

Compound [CAS Reg. No.]	Chemical name	Experimental activity [a]				
		SAL	YE3	L5T	REW	REP
Insecticides						
1. Acephate [30560-19-1]	*O,S*-Dimethyl *N*-acetylphosphoramidothiolate	+	+	+	NT	NT
2. Allethrin [584-79-2]	3-Allyl-2-methyl-4-oxo-2-cyclopentenyl[ ]-*cis,trans*-chrysanthemate	+	−		−	NT
3. Aspon [3244-90-4]	Tetra-*n*-propyl dithiopyrophosphate	−	−		NT	NT
4. Azinphos-methyl [86-50-0]	*S*-(3,4-Dihydro-4-oxobenzo-*d*-1,2,3-triazin-3-ylmethyl) dimethylphosphorothiolothionate	−	+	+	NT	NT
5. Carbofuran [1563-66-2]	2,3-Dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate	−	−		NT	NT
6. Chlordimeform [6164-98-3]	*N'*-[4-Chloro-2-methylphenyl]-*N,N*-dimethyl-methanimidamide	−	−		−	NT
7. Chlorpyrifos [2921-88-2]	Diethyl 3,5,6-trichloro-2-pyridyl phosphorothioate	−	−		+	+
8. Chrysanthemic acid[10453-89-1]	2,2-Dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylic acid	−	−		+	−
9. Crotoxyphos [7700-17-6]	Dimethyl 2[*a*-methylbenzyloxycarbonyl]-1-methylvinyl phosphate	−	+	+	NT	NT
10. Cypermethrin [52315-07-8]	*a*-Cyano-*m*-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	−	−		NT	NT
11. Diazinon [333-41-5]	*O,O*-Diethyl *O*-[2-isopropyl-4-methyl-6-pyridinimyl] phosphorothionate	−	−		NT	NT
12. *o*-Dichlorobenzene [95-50-1]	1,2-Dichlorobenzene	−	−		−	+
13. *m*-Dichlorobenzene [541-73-1]	1,3-Dichlorobenzene	−	+		−	+
14. *p*-Dichlorobenzene [106-46-7]	1,4-Dichlorobenzene	−	−		−	−
15. Ethion [563-12-2]	*O,O,O',O'*-Tetraethyl-*S,S'*-methylene biphosphorodithioate	−	−		−	−
16. Ethyl chrysanthemate[97-41-6]	2,2-Dimethyl-3-(2-methyl-propenyl)cyclopropanethylcarboxylate	−	+		−	−
17. Fensulfothion [115-90-2]	*O,O*-Diethyl-*O*-[*p*-(methylsulfinyl)phenyl]phosphorothioate	−	−		NT	NT
18. Fenthion [55-38-9]	*O,O*-Dimethyl-*O*-[4-(methylthio)-*m*-tolyl]phosphorothioate	−	−		NT	NT
19. Fonofos [944-22-9]	*O*-Ethyl-*S*-phenyl ethyldithiophosphonate	−	−		NT	NT
20. Formetanate [22259-30-9]	*m*-[((Dimethylamino)methyleneamino)phenyl] methylcarbamate	−	−		−	−

TABLE 1 (continued)

Compound [CAS Reg. No.]	Chemical name	Experimental activity [a]				
		SAL	YE3	L5T	REW	REP
21. Malathion [121-75-5]	*S*-[1,2-bis(ethoxycarbonyl) ethyl] *O,O*-dimethyl phosphorothioate	–	–		–	–
22. Methomyl [16752-77-5]	Methyl *O*-(methylcarbamoyl) thiolacetohydroxamate	–	–		–	–
23. Methoxychlor [72-43-5]	2,2-Bis(*p*-methoxyphenyl)1,1, 1-trichloroethane	–	–		–	–
24. Methyl-parathion [298-00-0]	*O,O*-Dimethyl-*O*-4-nitrophenyl-phosphorothioate	–	+	+	NT	NT
25. Monocrotophos [6923-22-4]	*cis*-1-Methyl-2-methyl carbamoyl vinyl phosphate	+	+	+	NT	NT
26. Parathion [56-38-2]	*O,O*-Diethyl-*O*-(*p*-nitrophenyl) phosphorothioate	–	–		–	–
27. Permethrin [52645-53-1]	3-Phenoxybenzyl-2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropanecarboxylate	–	–		NT	NT
28. Phorate [298-02-2]	*O,O*-Diethyl-*S*-ethylmercaptomethyl dithiophosphate	–	–		–	–
29. Resmethrin [10453-86-8]	3-Furanmethanol, 5-benzyl-2,2-dimethyl-3-(2-methylpropenyl) cyclopropanecarboxylate	–	–		NT	NT
30. Sumithrin [26002-80-2]	3-Phenoxybenzyl chrysanthemate	–	–		NT	NT
31. Trichlorfon [52-68-6]	*O,O*-Dimethyl (1-hydroxy-2,2,2-trichloroethyl)phosphonate	+	+	+	NT	NT
Herbicides						
32. Bromacil [314-40-9]	5-Bromo-3-*sec*.-butyl-6-methyl-3-(1-methylpropyl)-2,4(1*H*,3*H*)-pyridimidinedione	–	–	+	–	–
33. Diallate [2303-16-4]	2,3-Dichlorcallyl *N,N*-diisopropylthiolcarbamate	+	+	+		
34. Dicamba [1918-00-9]	3,6-Dichloro-2-methoxybenzoic acid	–	–		+	+
35. Dinoseb [88-85-7]	2-(1-Methylpropyl)-4,6-dinitrophenol	–	–		+	+
36. Monuron [150-68-5]	*N'*-(4-Chlorophenyl)-*N,N*-dimethyl urea	–	–	+	–	–
37. Pentachlorophenol[87-86-5]	Pentachlorophenol	–	+		+	–
38. Propanil [709-98-8]	*N*-(3,4-Dichlorophenyl)-propanamide	–	–		+	–
39. Siduron [1982-49-6]	*N*-(2-Methylcyclohexyl-*N*-phenyl-urea	–	–		NT	NT
40. Simazine [122-34-9]	6-Chloro-*N,N'*-diethyl-1,3,5-triazine 2,4-diamine	–	–	+	NT	NT
41. Sulfallate [95-06-7]	2-Chloroallyl *N,N'*-diethyldithiocarbamate	+	–		–	–
42. Triallate [2303-17-5]	*S*-2,3,3-Trichloroallyl *N,N'*-diisopropylthiocarbamate	+	+	+		
43. Trifluralin [1582-09-8]	2,6-Dinitro-*N,N*-dipropyl-4-(trifluoromethyl)-benzenamine	–	–	–	–	–

TABLE 1 (continued)

Compound [CAS Reg. No.]	Chemical name	Experimental activity [a]				
		SAL	YE3	L5T	REW	REP
44. 2,4-D [94-75-7]	(2,4-Dichlorophenoxy)acetic acid	–	–		+	–
45. 2,4-DB [94-82-6]	4-(2,4-Dichlorophenoxy)butanoic acid	–	–		–	+
46. 2,4,5-T [93-76-5]	(2,4,5-Trichlorophenoxy)acetic acid	–	–		+	–
Fungicides						
47. Biphenyl [92-52-4]	1,1'-Biphenyl	–	–		NT	NT
48. Captan [133-06-2]	*N*-(Trichloromethylthio)-4-cyclohexene-1,2-dicarboximide	+	+	+	+	+
49. Folpet [133-07-3]	2-[(Trichloromethyl)thio]-1*H*-isoindole-1,3(2*H*)-dione	+	+	+	+	+
50. Mancozeb [8018-01-7]	Manganous-zinc-ethylenebis(dithiocarbamate	–	+		–	–
51. Maneb [12427-38-2]	Manganous-ethylenebis(dithiocarbamate	–	+		–	–
52. PCNB [82-68-8]	Pentachloronitrobenzene	–	–		NT	NT
53. *sec.*-Butylamine [13952-84-6]	*sec.*-Butylamine	–	–		–	+
54. Zineb [12122-67-7]	Zinc ethylenebisdithiocarbamate	–	+		–	–

[a] NT refers to the case where inconclusive responses were obtained in the corresponding assay; +, positive; –, negative.

When present, fragment I causes a chemical to be active in YE3 with almost 100% probability. It is a methoxyphosphinyl-type group that is found mainly in insecticides. This is consistent with the observation of Wild (1975) that the phosphorus moiety in organophosphates appears to be a good substrate for nucleophilic attack. This may cause phosphorylation of the DNA, which is an instance of DNA damage.

Another interesting observation may be drawn by comparing the activating fragment I and the inactivating fragment VI. If we accept the premise that activity in the YE3 test is linked to the presence of a phosphorus moiety (i.e. fragment I) then it appears that the oxygen atom of the P = O bond is required. Its substitution by a sulfur atom is found to prevent activity (i.e., fragment VI).

By using the presence of one of the activating fragments (i.e., I–IV as an indication of activity and the absence of fragments I–IV or the presence

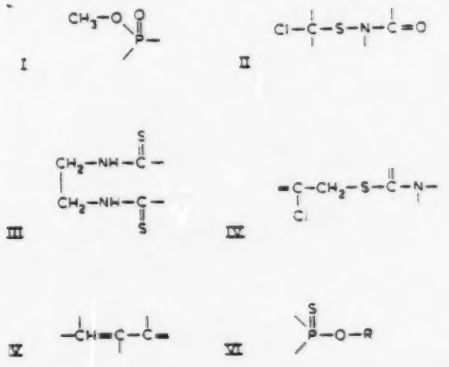

Fig. 1. Structure and statistical data of the most relevant descriptors of genotoxic activity in YE3, L5T and SAL. The probability values are related to the statistical significance of each subunit for activity or inactivity. Fragment I: activating probability 99.3%; fragment II: activating probability 91.5%; fragment III: activating probability 97.5%; fragment IV: inactivating probability 99.6%; fragment V: inactivating probability 99.0%; fragment VI: inactivating probability 90.2%.

TABLE 2

EXPERIMENTAL AND PREDICTED GENOTOXIC ACTIVITIES OF PESTICIDES IN THE YE3 TEST

Compound	Presence of selected fragments [a]							
	Activ.				Deactiv.		YE3 test	
	I	II	III	IV	V	VI	Obs.	Calc.
Acephate	1	0	0	0	0	0	+	+
Allethrin	0	0	0	0	0	0	−	(−)
Aspon	0	0	0	0	0	1	−	−
Azinphos-methyl	0	0	0	0	1	1	+	−
Carbofuran	0	0	0	0	0	0	−	(−)
Chlordimeform	0	0	0	0	1	0	−	(−)
Chlorpyrifos	0	0	0	0	1	1	−	(−)
Chrysanthemic acid	0	0	0	0	0	0	−	(−)
Crotoxyphos	1	0	0	0	0	0	+	+
Cypermethrin	0	0	0	0	0	0	−	(−)
Diazinon	0	0	0	0	0	1	−	−
m-Dichlorobenzene	0	0	0	0	0	0	+	(−)
o-Dichlorobenzene	0	0	0	0	1	0	−	−
p-Dichlorobenzene	0	0	0	0	0	0	−	(−)
Ethion	0	0	0	0	0	1	−	−
Ethyl chrysanthemate	0	0	0	0	0	0	+	(−)
Fensulfothion	0	0	0	0	0	1	−	−
Fenthion	0	0	0	0	1	1	−	−
Fonofos	0	0	0	0	0	1	−	−
Formetanate	0	0	0	0	0	0	−	(−)
Malathion	0	0	0	0	0	1	−	−
Methomyl	0	0	0	0	0	0	−	(−)
Methoxychlor	0	0	0	0	0	0	−	(−)
Methyl-parathion	0	0	0	0	0	1	+	−
Monococrotophos	1	0	0	0	0	0	+	+
Parathion	0	0	0	0	0	1	−	−
Permethrin	0	0	0	0	0	0	−	(−)
Phorate	0	0	0	0	0	1	−	−
Resmethrin	0	0	0	0	0	0	−	(−)
Sumithrin	0	0	0	0	0	0	−	(−)
Trichlorfon	1	0	0	0	0	0	+	+
Bromacil	0	0	0	0	0	0	−	(−)
Diallate	0	0	0	1	0	0	+	+
Dicamba	0	0	0	0	1	0	−	−
Dinoseb	0	0	0	0	1	0	−	−
Monuron	0	0	0	0	0	0	−	(−)
Pentachlorophenol	0	0	0	0	0	0	+	(−)
Propanil	0	0	0	0	1	0	−	−
Siduron	0	0	0	0	0	0	−	(−)
Simazine	0	0	0	0	0	0	−	(−)
Sulfallate	0	0	0	1	0	0	−	+
Triallate	0	0	0	1	0	0	+	+
Trifluralin	0	0	0	0	1	0	−	−
2,4-D	0	0	0	0	1	0	−	−
2,4-DB	0	0	0	0	1	0	−	−

TABLE 2 (continued)

Compound	Presence of selected fragments [a]							
	Activ.				Deactiv.		YE3 test	
	I	II	III	IV	V	VI	Obs.	Calc.
2,4,5-T	0	0	0	0	1	0	−	−
Biphenyl	0	0	0	0	1	0	−	−
Captan	0	1	0	0	0	0	+	+
Folpet	0	1	0	0	0	0	+	+
Mancozeb	0	0	1	0	0	0	+	+
Maneb	0	0	1	0	0	0	+	+
PCNB	0	0	0	0	0	0	−	(−)
sec.-Butylamine	0	0	0	0	0	0	−	(−)
Zineb	0	0	1	0	0	0	+	+

(−). No basis to support activity, the compound is presumed inactive.

[a] 1 or 0 refers to the presence/absence of the respective subunits, respectively.

of either fragment V or VI as an indication of inactivity), the results displayed in Table 2 were obtained. They indicate that 11 of the 16 compounds active in YE3 were correctly assessed and that 37 out of the 38 inactive molecules were identified correctly.

The same procedure was followed to find the most significant structural descriptors of activity in the SAL test. 5 fragments were automatically selected by the CASE program. The 3 activating fragments (I, II and IV) and both inactivating fragments (V and VI) are the *same subunits* responsible for activity or inactivity in YE3. This is noteworthy as it indicates a mechanistic relationship between the 2 tests. The presence of either fragment I, II or IV (used as an indication of activity), the absence of either fragment I, II or IV or the presence of fragments V or VI (used as an indication of inactivity) were the basis for the predictions summarized in Table 3. In this case, 8 out of the 9 active and all 45 inactive compounds were correctly identified.

Several attempts to correlate the chemical structure of pesticides with their mutagenicity in Salmonella have been reported previously (Carere et al., 1978; Wild, 1973; De Lorenzo et al., 1978). Carere et al. (1978) studied the mutagenicity of dichlorvos and other structurally related pesticides in Salmonella and Streptomyces. 6 of the pesticides tested by these authors in *S. typhimurium* (azinphosmethyl, diallate, monocrotophos, methylparathion, triallate and trichlorfon) were also present in our database. Carere and coworkers concluded that the presence of a chlorovinyl group, in these molecules, is responsible for the ability to induce point mutations in Salmonella. Fragment IV is consistent with this conclusion.

To test the quality of our descriptors of mutagenic activity in SAL, we obtained 2 compounds not included in the present database and reported by Carere et al. (1978) to display mutagenic activity in Salmonella. These compounds are dichlorvos (2,2-dichlorovinyldimethyl phosphate) and mevinphos (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate). They were analyzed by CASE and both were correctly predicted to be active in SAL.

The L5T test was employed by Waters et al. (1982) as a confirmatory assay for chemicals showing genetic activity in other systems. It was found that of 18 pesticides positive in YE3, 12 were also mutagenic in L5T. This led to the conclusion that L5T confirmed the activity of many chemicals detected in YE3. It was also observed that 10 pesticides displayed positive responses in SAL. Of these, 8 were positive in YE3 and also positive in L5T, leading to the conclusion that L5T confirms the activity of many pesticides in SAL (Waters et al., 1982). Our CASE analysis of the activity in SAL and YE3 assays is consistent with these observations (compare Tables 2 and 3). Indeed, a

TABLE 3

EXPERIMENTAL AND PREDICTED GENOTOXIC ACTIVITIES OF PESTICIDES IN THE SAL TEST

Compound	Presence of selected fragments [a]						
	Activ.			Deactiv.		SAL test	
	I	II	IV	V	VI	Obs.	Calc.
Acephate	1	0	0	0	0	+	+
Allethrin	0	0	0	0	0	+	(−)
Aspon	0	0	0	0	1	−	−
Azinphos-methyl	0	0	0	1	1	−	−
Carbofuran	0	0	0	0	0	−	(−)
Chlordimeform	0	0	0	1	0	−	−
Chlorpyrifos	0	0	0	1	1	−	−
Chrysanthemic acid	0	0	0	0	0	−	(−)
Crotoxyphos	1	0	0	0	0	−	(−)
Cypermethrin	0	0	0	0	0	−	(−)
Diazinon	0	0	0	0	1	−	−
m-Dichlorobenzene	0	0	0	0	0	−	(−)
o-Dichlorobenzene	0	0	0	1	0	−	−
p-Dichlorobenzene	0	0	0	0	0	−	(−)
Ethion	0	0	0	0	1	−	−
Ethyl chrysanthemate	0	0	0	0	0	−	(−)
Fensulfothion	0	0	0	0	1	−	−
Fenthion	0	0	0	1	1	−	−
Fonofos	0	0	0	0	1	−	−
Formetanate	0	0	0	0	0	−	(−)
Malathion	0	0	0	0	1	−	−
Methomyl	0	0	0	0	0	−	(−)
Methoxychlor	0	0	0	0	0	−	(−)
Methyl-parathion	0	0	0	0	1	−	−
Monocrotophos	1	0	0	0	0	+	+
Parathion	0	0	0	0	1	−	−
Permethrin	0	0	0	0	0	−	(−)
Phorate	0	0	0	0	1	−	−
Resmethrin	0	0	0	0	0	−	(−)
Sumithrin	0	0	0	0	0	−	(−)
Trichlorfon	1	0	0	0	0	+	+
Bromacil	0	0	0	0	0	−	(−)
Dialiate	0	0	1	0	0	+	+
Dicamba	0	0	0	1	0	−	−
Dinoseb	0	0	0	1	0	−	−
Monuron	0	0	0	0	0	−	(−)
Pentachlorophenol	0	0	0	0	0	−	(−)
Propanil	0	0	0	1	0	−	−
Siduron	0	0	0	0	0	−	(−)
Simazine	0	0	0	0	0	−	(−)
Sulfallate	0	0	1	0	0	+	+
Triallate	0	0	1	0	0	+	+
Trifluralin	0	0	0	1	0	−	−
2,4-D	0	0	0	1	0	−	−
2,4-DB	0	0	0	1	0	−	−

TABLE 3 (continued)

Compound	Presence of selected fragments [a]						
	Activ.			Deactiv.		SAL test	
	I	II	IV	V	VI	Obs.	Calc.
2,4,5-T	0	0	0	1	0	–	–
Biphenyl	0	0	0	1	0	–	–
Captan	0	1	0	0	0	+	+
Folpet	0	1	0	0	0	+	+
Mancozeb	0	0	0	0	0	–	(–)
Maneb	0	0	0	0	0	–	(–)
PCNB	0	0	0	0	0	–	(–)
sec.-Butylamine	0	0	0	0	0	–	(–)
Zineb	0	0	0	0	0	–	(–)

[a] See footnotes to Table 2.

complete overlap between the structural subunits responsible for activity or inactivity in YE3 and SAL is apparent. There is evidence for a common mechanism of action in inducing gene mutations in Salmonella (SAL) and recombination, i.e., DNA damage, in eukaryotes (YE3) for the pesticides under consideration.

To test the program's ability to reproduce the observed trends in a battery composed of SAL, L5T and YE3, the 13 compounds found to be active in L5T were evaluated using the descriptors of the YE3 and SAL databases. Table 4 indicates that activity in the battery (SAL, L5T, YE3) is essentially produced by 3 subunits, I, II and IV.

TABLE 4

EXPERIMENTAL AND PREDICTED GENOTOXIC ACTIVITIES OF PESTICIDES IN THE L5T TEST, AS PROJECTED BY EVALUATING THE COMPOUNDS ACTIVE IN L5T WITH THE DESCRIPTORS OF YE3 AND SAL

Compound	Presence of selected fragments [a]						
	Activ.			Deactiv.		L5T test [b]	
	I	II	IV	V	VI	Obs.	Calc.
Acephate	1	0	0	0	0	+	+
Azinphos-methyl	0	0	0	1	1	+	–
Crotoxyphos	1	0	0	0	0	+	+
Methyl-parathion	0	0	0	0	1	+	–
Monocrotophos	1	0	0	0	0	+	+
Trichlorfon	1	0	0	0	0	+	+
Bromacil	0	0	0	0	0	+	(–)
Diallate	0	0	1	0	0	–	+
Monuron	0	0	0	0	0	+	(–)
Simazine	0	0	0	0	0	+	(–)
Triallate	0	0	1	0	0	+	+
Captan	0	1	0	0	0	+	+
Folpet	0	1	0	0	0	+	+

[a] See footnotes to Table 2.

[b] This test was employed as a confirmatory assay in the experimental evaluation of pesticides genotoxicity (Waters et al., 1982).

Furthermore, by comparing the predicted activities displayed in Tables 2–4, the following observations may be drawn: of the 11 compounds correctly predicted to be active in YE3, 8 were also predicted to be active in L5T, which is in excellent agreement with the experimental confirmation index. Of the 8 compounds correctly predicted to be active in SAL, 7 were predicted to be active in L5T, which confirms the experimental observations cited above. As alluded to before, these observations lead to the unexpected conclusion that for the pesticides investigated, there is a *common basis for mutagenicity in bacteria (SAL) and mammalian cells (L5T)* even though their metabolism and the structure of their chromosomes may differ.

A CASE analysis of the activities of the pesticides in REW and REP was also performed. These 2 databases are small because many of the compounds tested in SAL and YE3 displayed ambigu-

TABLE 5

EXPERIMENTAL AND PREDICTED GENOTOXIC ACTIVITIES OF PESTICIDES IN REW

Compound	Presence of selected fragments [a]					
	Activ.			Deactiv.	REW test [a]	
	II	VII	VIII	IX	Obs.	Calc.
Allethrin	0	0	0	1	–	–
Chlordimeform	0	0	0	0	–	(–)
Chlorpyrifos	0	0	1	0	+	+
Chrysanthemic acid	0	1	0	0	+	+
m-Dichlorobenzene	0	0	0	0	–	(–)
o-Dichlorobenzene	0	0	1	0	–	–
p-Dichlorobenzene	0	0	0	0	–	(–)
Ethion	0	0	0	0	–	(–)
Ethyl chrysanthemate	0	0	0	1	–	–
Formetanate	0	0	0	1	–	–
Malathion	0	0	0	1	–	–
Methomyl	0	0	0	1	–	–
Parathion	0	0	0	0	–	(–)
Phorate	0	0	0	0	–	(–)
Bromacil	0	0	0	0	–	(–)
Dicamba	0	1	1	0	+	+
Dinoseb	0	1	0	0	+	–
Monuron	0	0	0	0	–	(–)
Pentachlorophenol	0	1	1	0	–	+
Propanil	0	0	1	0	+	–
Sulfallate	0	0	0	0	–	(–)
Trifluralin	0	0	0	0	–	(–)
2,4-D	0	1	1	0	+	+
2,4-DB	0	1	1	0	–	+
2,4,5-T	0	1	1	0	+	+
Captan	1	0	0	0	+	+
Folpet	1	0	0	0	+	+
Manozeb,	0	0	0	0	–	(–)
Maneb	0	0	0	0	–	(–)
sec.-Butylamine	0	0	0	0	–	(–)
Zineb	0	0	0	0	–	(–)

[a] See footnotes to Table 2.

ous response in these assays and were reported as 'no tests'. For REW, 3 activating subunits (II, VII and VIII) and 1 inactivating subunit (IX) were found to be the most relevant in determining activity or inactivity (Figs. 1 and 2). From these, the predictions displayed in Table 5 were obtained. It may be observed that 8 of the 10 active and all 22 inactive compounds were identified correctly. For REP, 3 activating fragments (II, X, XI) were required to identify correctly the activity of 25 of 28 compounds (Table 6). However, it would be presumptuous to assume direct causal relationship in this case because of the small size of the database.

The dichotomy in the structural features responsible for activity in REP and REW is unexpected. Both assays use bacteria to determine the preferential toxicity of DNA-damaging agents for DNA-repair deficient strains; one would have expected a common mechanism to be operative. A possible explanation for the observed differences is that REP uses *E. coli*, a gram-negative rod of the family Enterobacteriaceae, while REW uses *B. subtilis*, a gram-positive bacillus. It is known that

TABLE 6

EXPERIMENTAL AND PREDICTED GENOTOXIC ACTIVITIES OF PESTICIDES IN REP

Compound	Presence of selected fragments [a]				
	Activating			REP test [a]	
	II	X	XI	Obs.	Calc.
Chlorpyrifos	0	0	1	+	+
Chrysanthemic acid	0	0	0	−	(−)
m-Dichlorobenzene	0	1	0	+	+
o-Dichlorobenzene	0	1	0	+	+
p-Dichlorobenzene	0	0	0	−	(−)
Ethion	0	0	0	−	(−)
Ethyl chrysanthemate	0	0	0	−	(−)
Formetanate	0	1	0	−	−
Malathion	0	0	0	−	(−)
Methomyl	0	0	0	−	(−)
Parathion	0	0	0	−	(−)
Phorate	0	0	0	−	(−)
Dicamba	0	0	0	−	(−)
Dinoseb	0	0	1	+	+
Monuron	0	0	0	−	(−)
Pentachlorophenol	0	0	0	−	(−)
Propanil	0	0	0	−	(−)
Sulfallate	0	0	0	−	(−)
Trifluralin	0	0	0	−	(−)
2,4-D	0	0	0	−	(−)
2,4-DB	0	0	0	−	(−)
2,4,5-T	0	0	0	−	(−)
Captan	1	0	0	+	+
Folpet	1	1	0	+	+
Manozeb	0	0	0	−	(−)
Maneb	0	0	0	−	(−)
sec.-Butylamine	0	0	0	+	(−)
Zineb	0	0	0	−	−

[a] See footnotes to Table 2.

TABLE 7

PRESENCE OF SELECTED FRAGMENTS THROUGH THE 5 TEST SYSTEMS UNDER ANALYSIS [a]

Fragment	Test system				
	SAL	L5T	YE3	REW	REP
I	A	A	A	–	–
II	A	A	A	A	A
III	–	–	A	–	–
IV	A	A	A	–	–
V	In	In	In	–	–
VI	In	In	In	–	–
VII	–	–	–	A	–
VIII	–	–	–	A	–
IX	–	–	–	In	–
X	–	–	–	–	A
XI	–	–	–	–	A

[a] A, the corresponding fragment is present as an activating unit in the corresponding test system.
In, the corresponding fragment is present as an inactivating unit in the corresponding test system.
–, the fragment is not relevant for activity or inactivity in the corresponding test system.

the composition of the cell wall of *E. coli* facilitates the entry of lipophilic molecules, while the composition of the cell envelope of *B. subtilis* facilitates the entry of hydrophilic molecules. Conceivably, the observed dichotomy could reflect this

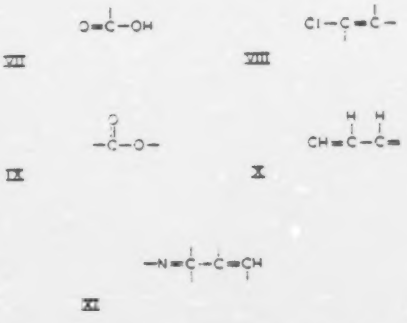

Fig. 2. Structure and statistical data of the most relevant descriptors of genotoxic activity in REW and REP. Also included in this set is the activating subunit II displayed in Fig. 1. Fragment VII: activating probability 96.0%; fragment VIII: activating probability 98.3%; fragment IX: inactivating probability 85.7%; fragment X: activating probability 90.8%; fragment XI: activating probability 90.4%.

difference. This could be approached experimentally by determining whether there is a difference in the partition coefficients between the structures responsible for the activity in REP and REW.

In view of the difference between REP and REW, one might ask why is the same phenomenon (i.e., differential solubility) not a factor when comparing SAL, which is a gram-negative bacterium, as test object with eukaryotic systems (YE3 and L5T). The answer probably derives from the fact that the composition of the cytoplasmic membrane of eukaryotes resembles in its lipophilicity that of the cell envelope of gram-negative microorganisms, thereby, not interfering with the recognition of the basic similarity of the chemical structures responsible for mutagenic and/or genotoxic activity.

Apart from the identification of the substructural features related to activity or inactivity in each biological system under study, perhaps the most interesting aspect of our analysis is the possibility of determining common structural factors responsible for the activity, or lack thereof, of a chemical in a given battery of short-term bioassays. The results of this battery are summarized in Table 7. As was mentioned earlier, subunits I, II and IV appear to be the common activating fragments for YE3, L5T and SAL. Similarly, subunits V and VI seem to be capable of preventing activity in this battery of tests. Thus, with respect to the pesticides, to test in each of these three assays may be redundant. It is also noteworthy that fragment II appears to be involved, as an activating subunit, in all 5 short-term bioassays (YE3, SAL, L5T, REW and REP), raising the possibility that it may be a particularly potent substructure.

Another interesting feature revealed by our analysis is the similarity between fragments V and X. Fragment V, which is an *ortho*-disubstituted aromatic fragment, inactivates chemicals in YE3, L5T and SAL. On the other hand, fragment X (the unsubstituted equivalent of fragment V) is an activating subunit in REP. The significance of the observation remains to be seen.

Conclusion

Relevant structural features have been identified that probably are responsible for activity or

inactivity in each category of genotoxic effects of the pesticides analyzed. Also, some interesting commonalities between the descriptors of genotoxic activity in the different short-term bioassays studied have been found. This information could be useful in further attempts to find structure–activity correlations involving molecular electronic indices. In turn, this could help to gain some knowledge about the mechanism of action of these chemicals. Despite the fact that the present database is not sufficiently complete to draw definitive relationships between genotoxic effects and chemical structure, some experimentally observed trends within the 5 test systems analyzed have been qualitatively reproduced. The 5 test systems fall into 2 categories of genetic activity with little commonality between then, and the results are consistent with the classification of Waters et al. (1982). The present study is unique in that it provides some information regarding the chemical features that differentiate these tests.

It should be noted that one of the strengths of the CASE methodology becomes apparent when the newly discovered relationship between the structural features responsible for the activity in the test systems studied herein are compared with the relationship found between the same assays as applied to large databases containing many classes of chemicals. The Gene-Tox database (Waters, 1979; Waters and Auletta 1981; Paladja and Rosenkranz, 1985) which contains information on the genotoxicity of approximately 2600 chemicals, permits a determination of the predictive relationship among test using Bayesian statistics (Rosenkranz et al., 1984; Chankong et al., 1985). Using that database, it can be calculated that a positive result in the SAL assay has a 57% chance of being positive in L5T and a positive result in REW has a 83% probability of being positive in SAL. Obviously, these statistical relationships do not hold for the subset of data analyzed herein and indicate that CASE will be most useful in elucidating common mechanistic features of subsets of chemical classes that are of environmental importance.

Moreover, CASE will also be of great help in designing a logical battery of assays that will detect features recognized as associated with activity. CASE permits the elimination of redundant assays and, therefore, will have an economical impact.

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